Effects of Surfactants on the GI Absorption of β-Lactam Antibiotics in Rats

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Abstract □ The effects of various nonionic and ionic surfactants and two bile salts (sodium cholate and sodium taurocholate) on the GI absorption of β-lactam antibiotics were investigated using the *in situ* rat GI perfusion technique. Addition of 10 m*M* polyoxyethylene-23-lauryl ether in the perfusion solution reduced the absorption of propicillin by the stomach and markedly increased the absorption of propicillin and cefazolin by the small intestine. Ester-type nonionic surfactants and bile salts exerted no significant influence on the intestinal absorption of these antibiotics.

Keyphrases □ Surfactants—effects on GI absorption of β-lactam antibiotics in rats □ Propicillin—effects of surfactants on GI absorption □ Cefazolin—effects of surfactants on GI absorption □ Absorption, GI—effects of surfactants, β-lactam antibiotics

Nonionic surfactants have often been employed in pharmaceutical preparations to formulate dispersed and solubilized systems, since they are less toxic to biological systems (1). It is known that drug–surfactant interactions modify the rate of intestinal absorption of drugs (2); some tend to enhance drug absorption and others tend to retard it.

GI absorption of nonabsorbed or poorly absorbed classes of β-lactam antibiotics can be promoted in rats and dogs by nonionic, anionic, and cationic surface-active agents. It was suggested that this promotion of antibiotic absorption was attributable to alteration of the membrane permeability of surfactants (3, 4). In previous studies (5, 6), nonionic and cationic surfactants were found to entrap penicillin molecules under pH conditions found in the stomach, resulting in remarkable stabilization and solubilization of penicillins. On the other hand, anionic surfactant micelles facilitated β-lactam cleavage. These results (3–6) indicated that acid-labile penicillins coated with relatively nontoxic nonionic surfactant micelles may increase the bioavailability after oral administration. The present study was undertaken to determine the effects of anionic, cationic, and nonionic surfactants, as well as bile salts on the GI absorption of β-lactam antibiotics using the *in situ* rat GI perfusion technique.

EXPERIMENTAL

Materials—Propicillin potassium[1] (993 μg/mg) and cefazolin sodium[2] (966 μg/mg) were used as received. Most surfactants employed were supplied by a commercial source[3]. Polyoxyethylene-23-lauryl ether (I), cetyltrimethylammonium bromide (II), and sodium lauryl sulfate (III) were the same as used in a previous study (6). Sodium taurocholate and sodium cholate were obtained commercially[4]. All other chemicals were of reagent grade and used without further purification.

***In Situ* GI Absorption Procedures**—Male albino rats of the Wistar strain, weighing ~200 g, were fasted overnight (20 hr) prior to the experiments, with water freely available. The rats were anesthetized 1 hr prior to surgery with urethan (1.3 g/kg ip). The procedures for rat surgery and absorption experiments using the GI recirculating method were those reported previously (7–9). The small intestine employed was a 30-cm length from the pylorus. The stomach or small intestine was washed with 50 ml of isotonic buffer solution of the desired pH, and then with 10 ml of the same buffer solution containing an appropriate concentration of surfactant. The drug solution was prepared using the buffer–surfactant solution maintained at 37° and perfused in a recirculating fashion with a pump at a rate of 10 ml/min for the stomach and 2 ml/min for the intestine. Unless otherwise stated, the antibiotic concentrations were 1 and 5 mg/ml for the stomach and intestinal experiments, respectively. The perfusion was maintained at the desired pH with a pH-stat[5] during the absorption experiments. The perfusion periods were 3 and 2 hr for the stomach and intestinal experiments, respectively.

At the end of the absorption experiments, the recirculating drug solution and the isotonic buffer solution perfused to wash the GI tract were collected into an appropriate volumetric flask, and the required amount of the same buffer solution was added to achieve the desired volume. To obtain the final sample, the solution was filtered through a 0.45-μm membrane filter[6]. The initial sample was prepared by dilution of un-

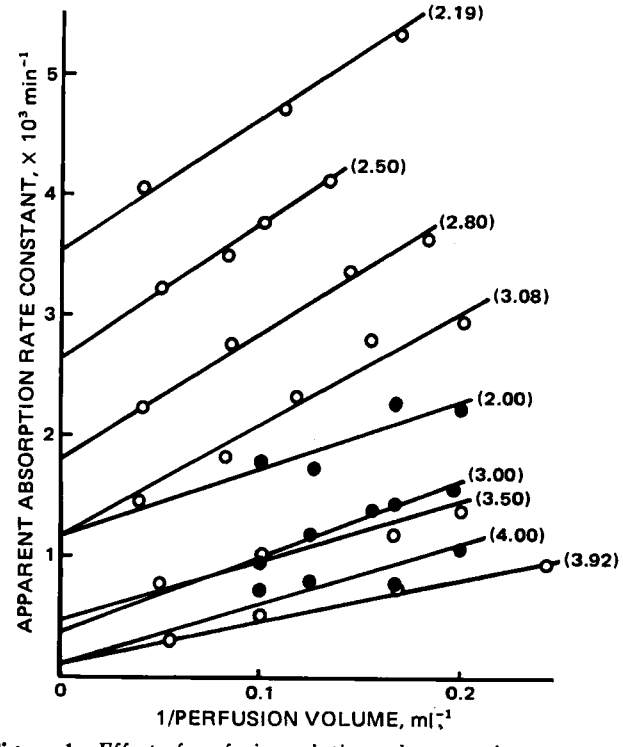

Figure 1—*Effect of perfusion solution volume on the apparent absorption rate constants (k_{app}) of propicillin from* in situ *rat stomach at various pHs (in parentheses) and 37°. The drug solution was perfused at a flow rate of 10 ml/min and the pH of the solution was maintained constant with a pH-stat. The points indicate experimental values: (●) in the presence of 10 mM I and (○) in the absence of I redrawn from Ref. 8.*

[1] Takeda Chemical Industries, Osaka, Japan.
[2] Fujisawa Pharmaceutical Co., Osaka, Japan.
[3] Nikko Chemicals Co., Tokyo, Japan.
[4] Wako Pure Chemical Industries, Osaka, Japan.
[5] pH-Stat titrator assembly consisting of a TTT2 titrator and ABU12b autoburette, Radiometer, Copenhagen, Denmark.
[6] Sartorius-membranfilter, GmbH, 34 Göttingen, West Germany.

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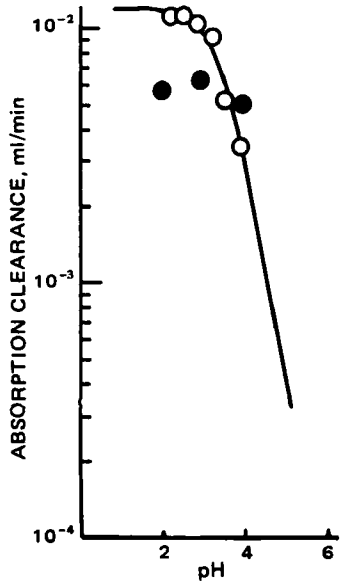

Figure 2—*Plots of the* in situ *rat stomach absorption clearance* CL_a *of propicillin* versus *the pH of the perfusion solution. The points indicate experimental values: (●) in the presence of 10 mM I and (○) in the absence of I redrawn from Ref. 8.*

perfused drug solution with isotonic buffer solution to the same volume.

The apparent first-order rate constants for the drug disappearance from the perfusate, k_{app}, were calculated as follows:

$$k_{app} = -\frac{1}{t}\ln\frac{C_t}{C_0} \qquad \text{(Eq. 1)}$$

where t represents the perfusion period and C_t and C_0 represent the drug concentrations in the final and initial samples, respectively.

Analytical Procedures—Propicillin and cefazolin in samples from the *in situ* absorption experiments were determined by reverse-phase high-performance liquid chromatography (HPLC) under the following conditions. The liquid chromatograph[7] was equipped with a UV detector[8] set at 254 nm. The stationary phase, made by chemically bonding a octadecylsilanol group to totally porous silica gel, was prepacked into a stainless steel column[9,10]. The mobile phases were 10 and 30% (v/v) acetonitrile–0.01 *M* ammonium acetate for cefazolin and propicillin, respectively. The instrument was operated at ambient temperature and at a flow rate of 1 ml/min. Samples were injected *via* a 100-μl loop injector[11] on flow. Peak heights were used for quantification.

During the *in situ* intestinal absorption experiments, 0.2-ml aliquots of blood were taken periodically from the jugular vein and assayed by the microbiological paper disk method employing *Salutina lutea*[12] as a test organism, after being hemolyzed with an equivalent volume of distilled water. The standard was established by employing pooled fresh blood from control rats.

The lower limit of sensitivity was 10 μg/ml for the HPLC assay and 1 μg/ml for the microbiological assay. The coefficients of variation ($SD \times 100$/mean) of the HPLC and microbiological assays throughout the analytical experiments were 3 and 5%, respectively, from many trials using standard solutions of each antibiotic.

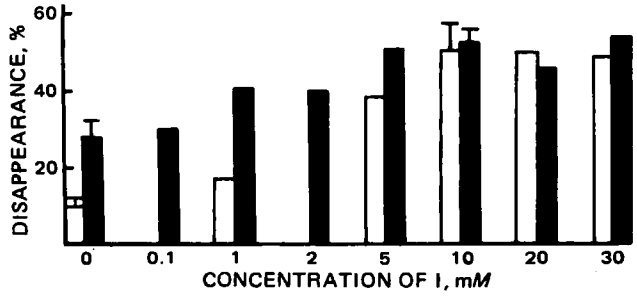

Figure 3—*Percentage disappearance of propicillin (■) and cefazolin (□) from the rat intestinal lumen as a function of the concentration of I. The perfusion solution was recirculated at a rate of 2 ml/min at pH 7.4, maintained constant with a pH-stat.*

[7] Model FLC-A700, Japan Spectroscopic Co., Ltd., Tokyo, Japan.
[8] Model UVIDEC-100, Japan Spectroscopic Co., Ltd., Tokyo, Japan.
[9] SC-01 (12.5 cm long × 4.6-mm i.d. column), Japan Spectroscopic Co., Ltd., Tokyo, Japan.
[10] μ-Bondapak C_{18} (30 cm long × 3.9-mm i.d. column), Waters Associates, Milford, Mass.
[11] Model LP1-350, Japan Spectroscopic Co., Ltd., Tokyo, Japan.

Table I—Percentage Disappearance of Cefazolin and Propicillin from *In Situ* Rat Small Intestine in the Presence of Various Surfactants and Bile Salts at pH 7.4 and 37°

Surfactant	HLB[a]	Concentration	Disappearance of Drugs, % (±*SD*)	n[b]
Cefazolin[c]				
None			9.7(1.8)[d]	3
Polyoxyethylene-10-oleyl ether	14.0	10 m*M*	52.8(12.1)	3
Polyoxyethylene-15-oleyl ether	16.0	10 m*M*	88.6	1
Polyoxyethylene-20-oleyl ether	17.0	10 m*M*	79.8	1
Polyoxyethylene-10-polyoxypropylene-4-cetyl ether	10.5	2% (w/v)	45.5(21.7)	3
Polyoxyethylene-20-polyoxypropylene-4-cetyl ether	16.5	2% (w/v)	65.4(11.7)	3
Polyoxyethylene-60-hydrogenated castor oil	14.0	1 m*M*	5.9	1
		5 m*M*	19.0(1.4)	3
		10 m*M*	14.4(3.3)	3
Polyoxyethylene-20-sorbitan monooleate	15.0	1% (w/v)	29.4	1
		2% (w/v)	4.9	1
Glyceryl monostearate	4.5	10 m*M*	22.7	1
Polyoxyethylene-20-glycerin vegetable oil fatty acid ester	15.5	2% (w/v)	16.1	1
Polyoxyethylene-15-glyceryl monostearate	13.5	10 m*M*	16.2	1
Polyoxyethylene-10-monooleate	11.0	5 m*M*	3.8	1
		10 m*M*	2.8	1
Polyoxyethylene-10-monolaurate	12.5	10 m*M*	19.2(10.1)	3
Polyoxyethylene-20-sorbitan monolaurate	16.9	10 m*M*	14.3	1
III		1 m*M*	5.0	1
		5 m*M*	48.2	1
		10 m*M*	52.1(6.9)	3
		20 m*M*	64.1	1
		30 m*M*	70.8	1
Sodium cetyl sulfate		1 m*M*	11.6	1
		2.5 m*M*	12.5	1
Sodium di-2-ethylhexyl sulfosuccinate		10 m*M*	28.2	1
Sodium taurocholate		10 m*M*	12.0(5.7)	5
Sodium cholate		10 m*M*	14.1(4.4)	5
Propicillin[c]				
None			28.4(4.1)[d]	3
II		10 m*M*	28.0(4.4)	5

[a] Hydrophilic–lipophilic balance. [b] Number of experiments. [c] The initial antibiotic concentration was 5000 μg/ml. [d] The data were taken from Ref. 8.

RESULTS

Effects of I on Stomach Absorption—The total disappearance of propicillin from the stomach perfusion solution has been reported to follow first-order kinetics (8). In this case, the apparent first-order rate constant undoubtedly includes the rate constants for both acid-catalyzed degradation and absorption, which proceed competitively during the absorption experiments.

As verified previously (7, 8), the apparent first-order rate constant, k_{app}, can be expressed as:

$$k_{app} = CL_a\frac{1}{V} + k_d \qquad \text{(Eq. 2)}$$

where CL_a is the absorption clearance in milliliters/time, k_d is the first-order rate constant for chemical degradation, and V is the volume of re-

[12] IFO 12708, Institute for Fermentation, Osaka, Japan; the strain was derived from ATCC 9341.

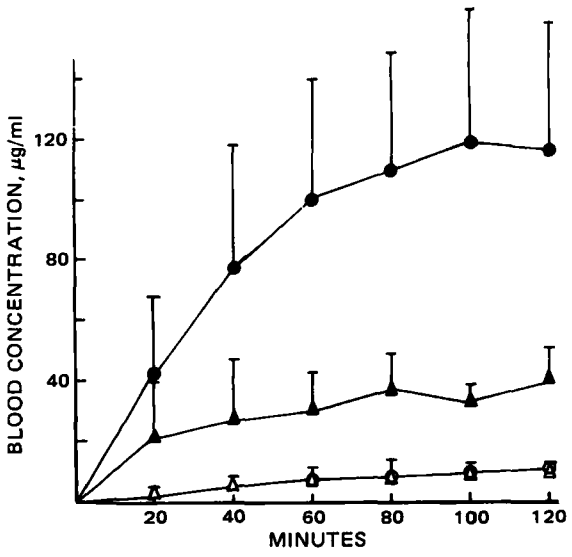

Figure 4—*Mean blood concentrations of propicillin (▲) and cefazolin (●) during* in situ *intestinal perfusion at pH 7.4 in the presence of 10 mM I. Open symbols indicate the blood concentrations in the absence of I. The vertical bars represent* SD *from three or more experiments.*

circulating drug solution. As shown in Fig. 1, plots of k_{app} obtained from absorption experiments with propicillin containing 10 m*M* I in the perfusion solution *versus* $1/V$ yielded a straight line similar to those obtained previously (8) for control experiments in the absence of I. The intercepts of these lines provided k_d values (in min^{-1}), which were evaluated to be 1.06×10^{-3}, 0.40×10^{-3}, and 0.30×10^{-3} at pH 2.00, 3.00, and 4.00, respectively.

It was found that the degradation of propicillin was much slower in the presence of the nonionic surfactant, which is consistent with the previous observations (5, 6). The CL_a of propicillin calculated from the slopes in Fig. 1 is plotted in Fig. 2 as a function of the perfusion solution pH. In this figure, the log CL_a–pH profile for propicillin determined under the same conditions in the absence of I was redrawn from our previous study (8). The absorption of propicillin below pH 3 was inhibited twofold in the presence of 10 m*M* I.

Effect of Surfactants on Intestinal Absorption—*Dependency on Concentration of I*—The effect of I on the intestinal absorption of two β-lactam antibiotics possessing different physicochemical characteristics was investigated. Propicillin was chosen since it has a high lipophilicity (10) and shows some incorporation (5, 6) of its ionized species into micelles of I; cefazolin has a low lipophilicity and exhibits negligible interaction with the surfactant micelles (5, 6).

Since the values of pK_a for the carboxylic acid groups of propicillin and cefazolin are 2.76 (10) and 2.54 (11), respectively, both antibiotics were largely in the form of anion at pH 7.4 in the present absorption experiments. Figure 3 shows the percentage disappearance of both antibiotics after recirculation through the small intestine for 2 hr as a function of the concentration of I. The total percentage disappearance for propicillin and cefazolin were determined previously (9) to be 28.4 ± 4.1 and 9.7 ± 1.8%, respectively. It was also found (9) that the greater disappearance of propicillin could be attributed mostly to intestinal degradation rather than absorption, and that the first-order rate constant ($\times 10^3$ min^{-1}) for the intestinal absorption was 1.29 ± 0.34 for propicillin and 0.85 ± 0.16 for cefazolin.

The relationship between the surfactant concentration and rate of antibiotic absorption represented one important aspect of the action of the surfactants. In the presence of I, the percentage disappearance of both antibiotics first increased markedly with increasing surfactant concentration ≤10 m*M* and then approached a constant value of ~50% >10 m*M*, which is much higher than the critical micellar concentration of 0.092 m*M* under comparative conditions (5, 6). For both antibiotics, no acceleration of the *in vitro* degradation in the presence of I was observed under the present experimental conditions, suggesting that the enhanced disappearance was a result of the absorption.

During the recirculating absorption experiments, the blood concentrations of propicillin and cefazolin were determined in the presence or absence of 10 m*M* I. The results are shown in Fig. 4 and clearly indicate that the presence of I remarkably increased the blood levels of both antibiotics. The accumulation of cefazolin in the gut tissue was only 0.2% after 2 hr. These findings strongly suggest that the amount of antibiotic disappearing from the intestinal perfusate in the presence of I was largely transferred to the serosal site.

Table II—Influence of Perfusion Solution pH on the Percentage Disappearance of Cefazolin from Rat Small Intestine in the Presence of 10 m*M* I Surfactant at 37°

pH	Disappearance, % (±*SD*)	*n*[a]
4.0	63.4(11.5)	3
4.7	50.4(5.8)	3
6.4	52.9(15.3)	3
7.4	50.7(6.6)	5

[a] Number of experiments.

Effect of Various Surfactants and Bile Salts—The promotional effect of surfactants on the intestinal absorption was investigated using concentrations of various surfactants and bile salts. The results are summarized in Table I. Promoted absorption by the surfactants was observed for both the anionic surfactant and the ether-type class of nonionic surfactants. Bile salts revealed no significant promotional effect. For nonionic surfactants used in this study, the ester groups showed a reduced or insignificant effect on cefazolin absorption.

Dependency on Intestinal Solution pH and the Initial Concentration of Drug—Absorption of cefazolin from the rat small intestine was examined in the presence of 10 m*M* I as a function of the perfusion solution pH. The results are shown in Table II. Between pH 4 and 7.4, there was no significant difference in the absorption behavior. Figure 5 gives logarithmic plots of the residual cefazolin concentration in the perfusate containing 10 m*M* I *versus* time for three different initial concentrations (1, 10, and 50 m*M*) at pH 7.4. The observed pseudo first-order rate constants were almost identical and independent of the initial drug concentration.

DISCUSSION

Davis *et al.* (3, 4) described the effects of surfactants on the GI absorption of poorly absorbed β-lactam antibiotics in dogs or rats, and attributed the promoted absorption by surfactants to alteration of the membrane permeability. In the present study, it was found that the absorption of propicillin from the rat stomach was reduced in the presence

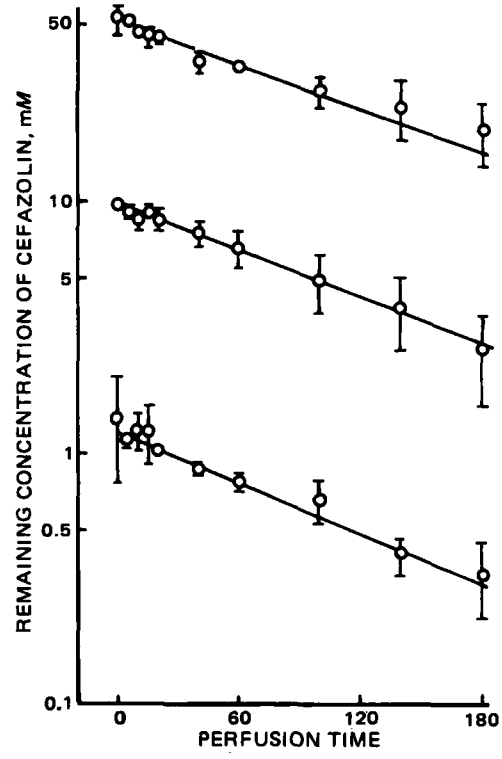

Figure 5—*Plots of the remaining cefazolin concentration in the perfusate* versus *the recirculating time at 37° and pH 7.4, maintained constant with a pH-stat. The perfusion solution was recirculated at a rate of 2 ml/min.*

of 10 m*M* I. Such reduction was interpreted as the result of entrapment of propicillin molecules into micelles of I. A similar phenomenon has been reported in absorption experiments with other drugs (12, 13). While the absorption of antibiotics by the small intestine increased in the presence of surfactants, such promoted absorption depended on the nature of the surfactants rather than the physicochemical properties of the antibiotics. Interestingly, the existence of a significant interaction between antibiotics and surfactants appeared not to influence the absorption by the small intestine. Despite the entrapment of 90% of the anionic propicillin into micelles of II (5, 6), no reduced absorption was observed (Table I). In the presence of 10 m*M* I, absorption of the antibiotic was promoted with 30% of the anionic species incorporated into the micelles. These results suggest that alteration of the membrane permeability by nonionic and cationic surfactants induced the promoted absorption in the small intestine, concurring with the findings made by other laboratories (3, 4).

Walters and Dugard (14) demonstrated that the hydrophilic–lipophilic balance (HLB) of a surfactant represents an important property in determining the promoted absorption. However, our results from the intestinal experiments indicated that significant absorption enhancement was observed only with ether-type surfactants, suggesting that HLB cannot be the sole cause for the observed promotional effect in the intestinal absorption of β-lactam antibiotics. Alteration of the permeability sometimes can be a result of disruption of the membrane structure by surfactants (15–17). In the present study, however, no significant disruption of the membrane was detected by light microscopy. The change in membrane permeability with the surfactant, therefore, may reflect a reversible alteration of the diffusion barrier to lipid soluble, poorly ionized drugs as claimed by Davis *et al.* (3, 4).

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Acrylic Microspheres *In Vivo* VI: Antitumor Effect of Microparticles with Immobilized L-Asparaginase Against 6C3HED Lymphoma

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Abstract □ The antitumor effect of immobilized L-asparaginase was tested against lymphoid leukemia in mice with concomitant scanning of the L-asparagine level in serum. L-Asparaginase was immobilized in microspheres of polyacrylamide or polyacryldextran. These particles were used in C3H mice bearing the L-asparagine-dependent lymphoma (6C3HED). The tumor was maintained as an ascites tumor, 1×10^6 cells were injected intraperitoneally and on day 4 after inoculation, L-asparaginase was injected intramuscularly or intraperitoneally in microparticles. After injection of 5.0 IU ip of L-asparaginase in microparticles, partial remission was induced, generally, however, the cancer relapsed and killed the mice within 2–3 weeks. To obtain complete regression, it was necessary to inject 20 IU of L-asparaginase in microparticles intraperitoneally. The best therapeutic effect was obtained when the particles were administered intramuscularly. After injection of 5 IU the survival time was prolonged, but complete regression was not achieved. The best effect was obtained when the particles were given intramuscularly in two small doses (2.5 IU) at a 3-day interval. Such treatment induced complete regression; 10 out of 12 treated mice were completely cured and lived for several months. It is concluded that the L-asparagine level in serum has to be depressed to <20% of the normal level for at least 6–7 days to obtain complete regression of the tumor.

Keyphrases □ Acrylic microspheres—*in vivo*, antitumor effect of microparticles with immobilized L-asparaginase against lymphoma □ L-asparaginase—acrylic microspheres, *in vivo*, antitumor effect of microparticles with immobilized L-asparaginase against lymphoma □ Antitumor effect—acrylic microspheres, *in vivo*, microparticles with immobilized L-asparaginase against lymphoma

L-Asparaginase of bacterial origin has been used extensively during the last 10 years in the treatment of lymphatic leukemia (1–3) as either a complement to or in combination with chemotherapy. The remission of the tumors is dose dependent (3) and considered to be due primarily to the deprivation of the cells of L-asparagine

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